Osteoarthritis and Cartilage (2001) **9,** 289–293

doi:10.1053/joca.2000.0387, available online at http://www.idealibrary.com on IDEAL®
1063–4584/01/040289+05 $35.00/0

Osteoarthritis and Cartilage
Journal of the OsteoArthritis Research Society International

Commentary

Recommendations for the registration of drugs used in the treatment of osteoarthritis: an update on biochemical markers

E. Vignon*, P. Garnero†, P. Delmas‡, B. Avouac§, P. Bettica‖, M. Boers¶, E. Ehrich**, N. MacKillop††, L. Rovati‡‡, U. Serni§§, T. Spector‖‖ and J. Y. Reginster¶¶ for the Group for the Respect of Ethics and Excellence in Science (GREES): Osteoarthritis Section

**Department of Rheumatology, Claude Bernard University, CHLS, 69310 Pierre Benité, France*
†*SYNARC, 13, rue Guilloud, 69003 Lyon, France*
‡*Department of Rheumatology, HEH, 69003 Lyon, France*
§*Department of Rheumatology, Hôpital Henri Mondor, 94010 Creteil, France*
‖*Procter & Gamble Pharmaceuticals, Staines, U.K.*
¶*Department of Clinical Epidemiology, University Hospital, Amsterdam, The Netherlands*
***Merck Pharmaceutical Inc., Rahway, New Jersey, U.S.A.*
††*Pfizer Central Research, Sandwich, U.K.*
‡‡*Rotta Research Laboratorium, Via Valosa di Sopra 7/9, 20052 Monza, Italy*
§§*Division of Rheumatology, Instituto Ortopedico Toscano, Florence, Italy*
‖‖*Twin Research & Genetic Epidemiology Unit, St Thomas Hospital, London, U.K.*
¶¶*WHO collaborating Center for Public Health Aspects of Rheumatic Disorders, University of Liège, Liège, Belgium*

Osteoarthritis (OA) is presently one of the rapidly expanding areas of research in the field of rheumatic diseases. This is evidenced by the ever increasing number of publications concerning the clinical, structural and biochemical manifestations of the disease. Effective medical treatment of OA is now emerging as a plausible eventuality. Distinction between symptom modifying OA drugs and structure modifying OA drugs (STMOAD) has been recommended[1,2]. Evaluation of STMOADs presents challenging and exciting clinical trial methodologic issues. Guidelines for clinical and radiographical evaluation in such trials have been developed[1–5]. Biochemical markers of OA is another rapidly developing field. The possibility of using such markers as diagnosis or prognosis factors for OA, as well as a method for assessment of progression of OA structural changes has been proposed. As such, biochemical markers may facilitate or enhance clinical evaluation of STMOADs.

In 1996 the Osteoarthritis (OA) Section of the Group for the Respect of Ethics and Excellence in Science (GREES) published the first guidelines for the registration of drugs used in the treatment of OA[2]. The quality and importance of this first work performed by the experts of the GREES was acknowledged by the Committee for Proprietary Medicinal Products (CPMP) at the European Agency for Evaluation of Medicines, which endorsed a number of their suggestions. The issue of biochemical markers was only briefly mentioned in the first GREES report. Thus, a working party of the GREES convened in Vienna in September 1999 to discuss whether biochemical markers of OA were applicable to: (1) be used as surrogates for radiography for the diagnosis of OA; (2) identify healthy individuals at risk for the development of OA; (3) predict the outcome of OA in the early stages of the disease in untreated or treated patients, (4) assess progression of OA in untreated or treated patients.

Address correspondence and reprint requests to: Pr Eric Vignon, Service de rhumatologie, Centre hospitalier Lyon-Sud, 69495 Pierre-Benité cedex, France. Tel: 04.78.86.12.20; Fax: 04.78.86.65.40; E-mail: eric.vignon@chu-lyon.fr

Proposed biochemical markers of OA

OA is a structurally complex disease that comprises cartilage destruction, subchondral bone sclerosis and cysts, osteophytes and synovial inflammation. Cartilage damage leading to progressive destruction of joint structure is generally considered to be the most important lesion of OA and most attention has been focused on this tissue. However, markers of other joint tissues are also being investigated as markers of OA.

Proteoglycans (PGs) and type II collagen are the major constituents of cartilage. Various antibodies recognizing nonspecified PG fragments, fragments containing either keratan sulfate (5D4, AN9P1) or chondroitin sulfate (3-B-3(-), 7-D-4, 846) and epitopes from the core protein

Table I
Potential biochemical markers of osteoarthritis

Markers	Tissue specificity	Specificity
Collagen N/C propeptides	Bone (type I) Cartilage (type II) Synovium (type I, III)	Formation
Pyridinium cross-links	None	Degradation
Collagen N/C telopeptide	Bone (type I) Cartilage (type II) Synovium (type I, III)	Degradation
Type II collagen α chains collagenase epitopes	Cartilage	Degradation
Bone alkaline phosphatase	Bone	Formation
Osteocalcin	Bone	Formation
Bone sialoprotein	Bone (subchondral)	Mineralization ?
Proteoglycan fragments	Cartilage ?	Degradation ?
Keratan sulfate (5D4, AN9P1)	Cartilage ?	Degradation ?
Chondroitin sulfate (3B3, 846)	Cartilage ?	Formation ?
COMP	None	Degradation ?
Hyaluronan	Synovium ?	Inflammation ?
YKL-4O	None	Synovium inflammation
CRP	None	Synovium inflammation
MMPs and TIMPs	None	Synovium inflammation ?

generated by metalloproteinases (MMPs) have been proposed as markers of PG turnover. Assays of collagen pyridinium cross-links, C/N telopeptides of type II collagen, neoepitopes generated by the collagenase cleavage of type II collagen and type II collagen propeptides (PII CP and type IIA N propeptide) have been developed as markers of cartilage collagen tunover. Other constituents of the cartilage, such as the cartilage oligomeric matrix protein (COMP), have also been suggested as markers of cartilage destruction.

Other proposed markers of OA include markers of synovial inflammation, such as C reactive protein (CRP), hyaluronan, YKL-40, metalloproteases (MMPs) and MMP inhibitors (TIMPs) and markers of bone turnover, such as pyridinoline and bone sialoprotein (BSP). No specific marker of osteophyte formation has yet been identified. Current and potential markers of OA are listed in Table I.

Validation of biochemical markers in osteoarthritis

The current list of potential or proposed markers of OA includes none that is truly specific for the disease. Instead, they reflect general remodeling of the various tissues of the joint, cartilage, synovium and bone.

Tissue specificity should be a primary characteristic of a biological marker. Some markers, such as bone alkaline phosphatase, osteocalcin and BSP, can be regarded as specific for bone turnover. Levels of D-pyridinoline and type I collagen peptides reflect predominantly bone turnover. Type II collagen markers are probably the most specific for cartilagineous tissues including meniscus and intervertebral discs. Hyaluronan is accepted as a marker of the synthetic activity of synovium. Other proposed markers such as proteoglycan fragments, pyridinoline, COMP, CRP, YKL-40, MMPs and TIMPs lack a clear specificity.

A meaningful biochemical marker must also specifically reflect either the synthesis or the degradation of a tissue. The specificity of markers for bone formation and bone degradation is now well accepted. C/N telopeptides of type II collagen and type II collagen propeptides, respectively, probably specifically reflect the degradation and the synthesis of cartilage collagen. The significance, in terms of formation/degradation, of other proposed markers of cartilage, such as proteoglycan epitopes and COMP is less clear.

To be applicable ('valid') in a certain setting, any measure must be truthful, discriminative between conditions of interest, and feasible in terms of cost, time and interpretability[6]. In terms of truthfulness, validation of a biochemical marker requires a good correlation between a marker level in a sample fluid and biologic activity in the tissue. Discrimination depends on the purpose of the measurement: distinguishing between disease and nondisease, assessing prognosis, or assessing change over time. Study of applicability is quite difficult, particularly for articular cartilage. It might be helpful to consider joint tissues as an organ and the synovial fluid (SF) as the biological medium of the organ, i.e. to correlate between SF and serum or urine findings. Assays and interpretation of markers in the SF are especially difficult and SF collection may often be technically or ethically difficult. Markers in SF are probably valuable for evaluation of what is happening in a target joint. Markers in blood and urine probably reflect what is happening in multiple joints. Site and number of joints to consider for accurate evaluation of blood and urine markers remain a difficult question.

An increase in serum or urine of patients with knee or hip OA has been reported for proteoglycan monomer fragments[7,8], 5D4[9–11], 3B3 and 846[12–15], COMP[11,16–20], C telopeptide and neoepitope of type II collagen[21,23], PII CP[24,25], hyaluronan[26–28], CRP[29,30], YKL-40[31–33], MMPs[34], BSP[30,35] and pyridinoline[36–38]. Decreased serum levels of ANP9 have been reported in patients with primary OA[11]. Conflicting results were obtained with pyridinoline[37,38]. Most of the data generally have been obtained from small cross-sectional studies. Thus, present knowledge of the role of OA markers is limited. Technical validation of a number of assays, as well as standardization or centralization of the assays will also be necessary.

Biochemical markers for identification and staging of OA patients

One of the problems in the study of markers is the fact that there is no gold standard for the diagnosis of OA. Current definitions of OA are based on clinical signs and/or radiographical lesions. Thus, a biochemical marker could be helpful to more precisely identify OA patients. As mentioned above, no OA specific epitope has yet been identified and proposed markers of OA are generic markers of joint tissue remodeling. For example, COMP has been proposed as a marker for an OA population[20]. However, overlap between patients and healthy controls is generally large enough to prevent the use of COMP or any current marker on an individual patient basis. Moreover increased levels of markers of cartilage, synovium or bone have been found in various joint diseases other than OA[39–42]. Thus, proposed markers cannot presently be considered as useful for identification of OA patients. Similar considerations and conclusions apply to the use of markers in identifying future OA patients in a healthy population.

The use of biochemical markers for the staging of OA patients is another question. Staging of OA can be made according to the degree of pain or impairment or to the degree of structural joint damage. Discordance between symptoms and radiography is well known and grading is generally based on structural change scores such as the Kellgren and Lawrence. Variation of proteoglycan epitope with joint space narrowing grade has been reported[15,43]. An Increase in urine pyridinium cross-links of collagen in a late X-ray stage of OA, a stage characterized by obvious subchondral bone changes, has also been suggested[36,38]. Synovium inflammation and hypertrophy is also known to progress with duration of the disease. Thus, some markers, alone or in combination, could possibly be helpful in the future for the staging of OA.

Biochemical markers as predictive factor for OA progression

The high prevalence of OA is well known. However, among patients with hip or knee OA only a relatively small proportion of them develop progressive disease with severe pain and disability, eventually justifying surgery. Identification of patients at risk for such progression is clearly of interest. The interest will probably increase in the near future for cost/benefit analysis of potential STMOADs. Increased levels of joint tissue markers, for cartilage degradation especially, should theoretically be helpful. A predictive value for progression has been suggested for COMP[17–19], hyaluronan and CRP[27,29,30]. However, long-term studies of cohorts evaluated with the use of modern validated clinical and radiographical methods remain to be performed.

Biochemical markers for the assessment of OA treatment

There is a potential utility of markers for the assessment of OA progression in treated patients. For instance, a marker of progressive cartilage destruction might allow more rapid and more sensitive evaluation of a chondroprotective treatment than is possible with radiographic measurements spanning several years. Conversely, a possible deleterious effect on cartilage degradation of a clinically effective treatment could also be detected more rapidly. Markers of other joint tissues could also be relevant. A correlation between BSP and OA progression has been suggested[35]. Again, validation of markers for such a purpose remains to be performed. This is a difficult task, necessitating accurate clinical and radiographical measurements in a large number of untreated patients and over a long period.

Conclusion

The present view on biochemical markers of OA might appear pessimistic. However, it reflects the fact that research in the field has only just started. Clearly, the group underlined the multiple potential applications of biochemical markers of OA. Assaying specific markers of cartilage degradation and synthesis, of bone remodeling and of synovial inflammation will constitute important tools in expanding our understanding of OA. At the present time, identification, among current proposed markers, of the best candidates is premature. Finally, the inclusion of patients in the placebo group of long-term therapeutic trials is essential to characterize the change of these markers over time. All together, these data are likely to be critical for validation of biochemical markers of OA.

References

1. Altman RD, Brandt K, Hochberg M, Moskowitz R. Design and conduct of clinical trials in patients with osteoarthritis: recommendations from a task force of the Osteoarthritis Research Society. Osteoarthritis Cart 1996;4:217–43.
2. Group for the Respect of Ethics and Excellence in Science (GREES). Recommendations for the registration of drugs used in the treatment of osteoarthritis. Ann Rheum Dis 1996;55:552–7.
3. Bellamy N, Kirwan J, Boers M, Brooks P, Strand V, Tugwell P. Recommendations for a core set of outcome measures for future phase III clinical trials in knee, hip and hand osteoarthritis. Consensus development at OMERACT III. J Rheumatol 1997;24:799–802.
4. Bellamy N. Clinical trials design: structure modifying agents for osteoarthritis. Future guidelines: areas for development. Osteoarthritis Cart 1999;7:424–6.
5. Vignon E, Conrozier T, Piperno M, Richard S, Carrillon Y, Fantino O. Radiographic assessment of hip and knee osteoarthritis. Recommendations: recommended guidelines. Osteoarthritis Cart 1999;7:434–6.
6. Boers M, Brooks P, Strand V, Tugwell P. The OMERACT filter for outcome measure in rheumatology. J Rheumatol 1998;25:198–9.
7. Lohmander LS, Dahlberg L, Ryd L, Heinegard D. Increased levels of proteoglycan fragments in knee joint injury. Arthritis Rheum 1989;32:1434–42.
8. Lohmander LS, Hoerrner LA, Lark MW. Metalloproteinases, tissue inhibitor, and proteoglycan fragments in knee synovial fluid in human osteoarthritis. Arthritis Rheum 1993;36:181–9.
9. Thonar EJ, Lenz ME, Kintworth GK, Caterson B, Patchman LM, Glickman P. Quantification of keratan sulfate in blood as a marker of cartilage metabolism. Arthritis Rheum 1985;28:1367–76.

10. Sweet MBE, Coelho A, Schnitzer CM, Schnitzer TJ, Lenz ME, Jaim I, *et al.* Serum keratan sulfate levels in osteoarthritis patients. Arthritis Rheum 1988;31: 648–52.
11. Bleasel JF, Poole AR, Heinegard D, Saxne T, Holderbaum D, Ionescu M, *et al.* Changes in serum cartilage marker levels indicate altered cartilage metabolism in families with the osteoarthritis-related type II collagen gene COL2A1 mutation. Arthritis Rheum 1999;42:39–45.
12. Poole AR, Ionescu M, Swan A, Dieppe PA. Changes in cartilage metabolism in arthritis are reflected by altered serum and synovial fluid levels of the cartilage proteoglycan aggrecan. Implications for pathogenesis. J Clin Invest 1994;94:25–33.
13. Belcher C, Yaqub R, Fawthriop F, Bayliss M, Doherty M. Synovial fluid chondroitin and keratan sulfate epitopes, glycosaminoglycans and hyaluronan in arthritic and normal knees. Ann Rheum Dis 1997;56: 299–307.
14. Ishiguro N, Ito T, Iwata H, Jugessur H, Ionescu M, Poole R. Relationship of matrix metalloproteinases and their inhibitors to cartilage proteoglycan and collagen turnover. Arthritis Rheum 1999;42:129–36.
15. Lohmander LS, Ionescu M, Jugessur H, Poole AR. Changes in joint cartilage aggrecan after knee injury and osteoarthritis. Arthritis Rheum 1999;42:534–44.
16. Lohmander LS, Saxne T, Heinegard DK. Release of cartilage oligomeric matrix protein (COMP) into joint fluid after knee injury and in osteoarthritis. Ann Rheum Dis 1994;53:8–13.
17. Sharif M, Saxne T, Shepstone L, Kirwan JR, Elson CJ, Heinegard D, *et al.* Relationship between serum cartilage oligomeric matrix protein levels and disease progression in osteoarthritis of the knee joint. Brit J Rheumatol 1995;34:306–10.
18. Conrozier T, Saxne T, Shan Sei Fan C, Mathieu P, Tron A-M, Heinegard D, *et al.* Serum concentrations of cartilage oligomeric matrix protein and bone sialoprotein in hip osteoarthritis: A one year prospective study. Ann Rheum Dis 1998;9:527–32.
19. Petersson IF, Boegard T, Dahlstrom J, Svensson B, Heinegard D, Saxne T. Bone scan and serum markers of bone and cartilage in patients with knee pain and osteoarthritis. Osteoarthritis Cart 1998;6: 33–9.
20. Clark AG, Jordan JM, Vilim V, Renner JB, Dragomir AD, Luta G, *et al.* Serum cartilage oligomeric matrix protein reflects osteoarthritis presence and severity. Arthritis Rheum 1999;42:2356–64.
21. Eyre DR, Shao P, Vosberg-Smith K, Weis M, Shaffer K, Yoshihara P. Cross-linked telopeptides from collagen types I, II and III in human urine. J Bone Miner Res 1995;S1:S413.
22. Moskowitz RW, Holderbaum D, Atley LM, Eyre DR. Type II C-telopeptide 2B4 epitope is a marker for cartilage degradation in familial osteoarthritis. Arthritis Rheum, 1998;41(Suppl.):S352.
23. Woodworth TG, Otterness IG, Johnson K, Pickering E, Saltarelli MJ. Urinary type II collagen neoepitope (uTIINE) in osteoarthritis (OA) is associated with disease severity. Arthritis Rheum, 1999;42(Suppl): S258.
24. Lohmander LS, Yoshihara Y, Roos H, Kobayashi T, Yamada H, Shinmei M. Procollagen II C-propeptide in joint fluid: changes in concentration with age, time after knee injury, and osteoarthritis. J Rheumatol 1996;23:1765–9.
25. Nelson F, Dahlberg L, Laverty S, Reiner A, Pidoux I, Ionescu M. Evidence for altered synthesis of type II collagen in patients with osteoarthritis. J Clin Invest 1998;12:2115–25.
26. Balblanc JC, Conrozier T, Hartmann D, Mathieu P, Richard M, Vignon E. Acide hyaluronique et phopholipase A2 sériques dans une population arthrosique. Rev. Rhum 1991;58:857–62.
27. Sharif M, George L, Shepstone J, Knudson W, Thonar EJ-MA, Cushnagan J, *et al.* Serum hyaluronic acid level as a predictor of disease progression in osteoarthritis of the knee. Arthritis Rheum 1995;38: 760–7.
28. Laurent TC, Larent UBG, Fraser RE. Serum hyaluronan as a disease marker. Ann Med 1996;28:241–53.
29. Spector TD, Hart DJ, Nandra D, Doyle DV, MacKillop N, Gallimore JR. MBLow-levels increases in serum C-reactive protein are present in early osteoarthritis of the knee and predict progressive disease. Arthritis Rheum 1997;40:723–7.
30. Conrozier T, Chappuis-Cellier C, Richard M, Mathieu P, Richard S, Vignon E. Increase C-reactive protein levels by immunonephelometry in patients with rapidly destructive hip osteoarthritis. Rev Rhum (Engl. Ed.) 1998;12:759–65.
31. Johansen JS, Jensen HS, Price PA. A new biochemical for joint injury. Analysis of YKL-40 in serum and synovial fluid. Brit J Rheumatol 1993;32:949–55.
32. Johansen JS, Hvolris J, Hansen M, Backer V, Lorenszen I, Price PA. Serum YKL-40 levels in healthy children and adults. Comparison with serum and synovial fluid levels of YKL-40 in patients with osteoarthritis or trauma of the knee joint. Brit J Rheumatol 1996;35:553–9.
33. Harvey S, Weisman M, O'Dell J, Scott T, Krusemeier M, Visor J, *et al.* Chondrex: a new marker of joint disease. Clin Chem 1998;44:509–16.
34. Naito K, Takahashi K, Kushida K, Suzuki M, Ohishi T, Miura M. Measurement of matrix metalloproteinases (MMPs) and tissue inhibitor of metalloproteinase-1 (TIMP-1) in patients with knee osteoarthritis: comparison with generalized osteoarthritis Rheumatology 1999;38:510–5.
35. Petersson IF, Boegard T, Svensson B, Heinegard D, Saxne T. Changes in cartilage and bone metabolism identified by serum markers in early osteoarthritis of the knee joint. Brit J Rheumatol 1998;37:46–50.
36. Thompson PW, Spector TD, James IT, Henderson E, Hart DJ. Urinary collagen crosslinks reflect the radiographic severity of knee osteoarthritis. Brit Med J 1992;31:759–61.
37. Astbury C, Bird HA, McLaren AM, Robin SP. Urinary excretion of pyridinium crosslinks of collagen correlated with joint damage in arthritis. Brit J Rheumatol 1994;33:11–15.
38. Hellio le Graverand MP, Tron AM, Dallard MC, Richard M, Uebelart D, Vignon E. Assessment of urinary hydroxypyridinium crosslink measurements in osteoarthritis. Brit J Rheumatol 1996;35:1091–5.
39. Saxne T, Heinegard D, Wollheim FA, Petterson H. Difference in cartilage proteoglycan level in synovial

fluid in early rheumatoid arthritis and reactive arthritis. The Lancet 1985;2:127–8.
40. Forslind K, Eberhardt K, Jonsson A, Saxne T. Increased serum concentration of cartilage oligomeric matrix protein. A prognostic marker in early rheumatoid arthritis. Brit J Rheumatol 1992;31: 593–8.
41. Mansson B, Carey D, Alini M, Ionescu M, Rosenberg LC, Poole AR, *et al.* Cartilage and bone metabolism in rheumatoid arthritis. Differences between rapid and slow progression of disease identified by serum markers of cartilage metabolism. J Clin Invest 1995; 95:1071–7.
42. Iwase T, Hasegawa Y, Ishiguro N, Ito T, Iwasada S, Kitamura S, *et al.* Synovial fluid cartilage metabolism marker concentrations in osteonecrosis of the femoral head compared with osteoarthrosis of the hip. J Rheumatol 1997;25:527–31.
43. Saxne T, Heinegard D. Synovial fluid analysis of two groups of proteoglycan epitopes distinguished early and late cartilage lesions. Arthritis Rheum 1992;35:385–90.